Supporting Information

SI Materials and Methods

Antibodies and reagents. Allophycocyanin (APC)- or phycoerythrin-Cy7 (PE/Cy7)-conjugated monoclonal anti-CD31 (WM59) (303117), anti-VEGF-R2 (HKDR-1) (338909), anti-CD34 (581) (343509), anti-Tie2 (33.1) (334209), anti-Endoglin (43A3) (323217), anti-NRP1 (12C2) (354505), anti-CXCR4 (12G5) (306513), anti-CD45 (HI30) (304015), control mouse IgG1 (MOPC-21) (400119 and 400125), 7-amino actinomycin (7-AAD) (all purchased from BioLegend), and anti-CD144 (16B1) (eBioscience) were applied to FACS-sorting and analysis. Primary antibodies applied to immunofluorescence analysis were anti-VE-cadherin (BV9) (348501), anti-mouse CD31 (MEC13.3) (102501) (1:200; both from BioLegend), anti-vWF (rabbit polyclonal) (ab9378), anti-eNOS (rabbit polyclonal) (ab5589) (1:100; both from abcam), anti-α-SMA (ERP5368) (GTX63570) (1:500; GeneTex), anti-human CD34 (QBEND/10) (MCA547GT) (1:400; AbD serotec), and anti-VE-cadherin (rabbit polyclonal) (LS-B2138) (1:200; Lifespan). Secondary antibodies were goat anti-mouse IgG-Alexa Fluor 546 (A11018) and goat anti-rabbit IgG-Alexa Fluor 633 (A21072) (1:1,000; both from Invitrogen). Rhodamine-conjugated UEA I (1:160) was purchased from Vector Laboratories. Primary antibodies applied to Western blot analysis were anti-ETV2 (EPR5229(3)) (1:500; abcam) and anti-β-actin (rabbit polyclonal) (A-2066) (1:1000; Sigma). Secondary antibody was goat anti-mouse IgG-horse radish peroxidase (A11018) (1:10000; Jackson ImmunoResearch).

Cell culture. Human embryonic lung fibroblast line HFL-1 cells (RCB0521), human neonatal skin fibroblast line NB1RGB cells (RCB0222) (both from RIKEN), and HAFs (C-013-5C) (Invitrogen) were expanded in α-MEM (Invitrogen) supplemented with 10% FBS (Invitrogen) and penicillin/streptomycin. Before being used for experiments, HAFs were passaged three times. HUVECs (KJB-110) (DS Pharma Biomedical) were grown in EGM-2 medium (Lonza) on 10-cm dishes coated with 100 μg/ml type I collagen (Nitta Gelatin). HEK293T cells (RCB2202) (RIKEN) were cultured in DMEM (Gibco) supplemented with 10% FBS, 2 mM L-glutamine, and penicillin/streptomycin. Human peripheral blood mononuclear cells (PBMCs) from healthy volunteers were obtained with written informed consent under an approved protocol of the ethical board at Keio University. All the cell lines were authenticated. Mycoplasma contamination was not detected in any of the cell lines or HAFs by PCR analysis.

Lentivirus production and transduction of cells. Human *ERG*, *FLI1*, *FOXC2*, *GATA2*, *HHEX*, *HOPX*, *HOXA9*, *KLF4*, *LMO2*, *MEF2A*, *MEIS1*, *RUNX1*, *SOX4*, *TAL1*, *TCF4*, *VEZF1* cDNAs were obtained from RT-PCR using human PBMCs, HUVECs, and human monocytic leukemia cell line THP-1 cells (RCB1189) (RIKEN). Human *ETV2* expression vector (pF1KB9707) was purchased from Kazusa DNA Research Institute. Human *HOXB4* expression vector was gifted by Y. Takihara and K. Humphries. HA-tagged *ETV2* mutants were generated by site-directed mutagenesis (1). The cDNAs were subcloned into lentivirus vector CSII-EF-MCS-IRES2-Venus (RIKEN). All of the coding sequences in the expression vector were confirmed with an ABI PRISM 310 Genetic Analyzer (ABI). For the inducible *ETV2* expression system, HA-tagged *ETV2* and reverse tetracycline transactivator (rtTA) were subcloned into lentivirus vector

under the control of the tetracycline operator and CSII-EF-MCS (RIKEN), respectively. For human *FOXC2*-knocking down experiments, pLKO5 that was gifted by S.A. Mani and pLKO.1-puro luciferase (Sigma) were used as a targeting and a control vector, respectively (2). To produce recombinant lentiviruses, the cDNA expression vector was transduced into HEK293T cells along with VSV-G expression vector pCMV-VSV-G-RSV-Rev (RIKEN) and packaging vector pMDLg/p-RRE. Eighteen hours after transduction, the vector-containing culture medium was changed into fresh culture medium and 48 h later, the lentivirus-containing medium was collected, passed through a 0.45-μm filter, and concentrated using centrifugation (8,400*g* at 4^{o}C for 16 h). The lentivirus pellets were resuspended in PBS. HFL-1 cells, NB1RGB cells, and HAFs were seeded on 12-well plates at 7 x 10^4 cells per well and 24 h later infected with the concentrated lentivirus particles with 5 μg/ml protamine. Another 48 h later, cells were washed twice with PBS and then cultured on 6-cm dishes coated with 100 μg/ml type I collagen in EGM-2 medium supplemented with 10 ng/ml recombinant human $VEGF_{165}$ (Peprotech) and bFGF (Wako). Doxycycline (Sigma) was used at 5 to 1000 ng/ml for inducing *ETV2* expression. Puromycin (Invivogen) was used at 2 μg/ml for selecting shRNA-expressing fibroblasts. For some experiments, 4 days after *ETV2* transduction, $Venus^+$ cells were sorted. Nine and 12 days after the transduction, 80% of the expanding cells were cryopreserved in CellBanker-1 Plus (Zenoaq) at -80^{o}C for the future culture, and the remaining cells continued in culture on 10-cm dishes coated with 100 μg/ml type I collagen. The culture medium was replaced every 2 or 3 days until day 36. Cells into which genes are transduced by the lentivirus vector were positive for Venus expression. Fifteen and 25 days after transduction, $CD31^+$ cells were sorted as described below. Cell morphology was recorded using a light microscope (Nikon).

Single cell culture. Venus^{+} HAFs at 4 days after *ETV2* infection were plated into 96-well flat-bottom plates coated with 100 μg/ml type I collagen at 1 cell per well. Eleven days later, cells were fixed with paraformaldehyde (PFA) (Wako) and then stained with rhodamine-conjugated monoclonal anti-CD31 (P2B1) (1:200; Santa Cruz Biotech. Inc.). The images were acquired with a BZ-8000 fluorescence microscope (Keyence) and analyzed using BZ-Analyzer software. CD31 expression of each clone was determined by a third person who was not informed the detailed purpose of this experiment.

Quantitative RT-PCR. Total RNA was extracted using an RNeasy Plus Micro Kit with a genomic DNA Eliminator (QIAGEN) and subjected to reverse transcription with a High Capacity cDNA Synthesis Kit (Applied Biosystems). PCR analysis was performed using a KAPA SYBR FAST qPCR Kit (KAPABIOSYSTEMS) on an iCycler iQ multicolor real-time PCR detection system (Bio-Rad) and SsoFast EvaGreen Supermix (Bio-Rad). Relative gene expression levels were normalized by comparison to *HPRT1*. Gene-specific primer pairs are listed in Table S4.

Dil-AcLDL uptake assay and immunofluorescence microscopy. Twenty-four hours before the assay, HAFs, ETVECs, and HUVECs were plated on a collagen I-coated 8-well slide (BD Biosciences) at 4 x 10^4 cell per well in EGM-2 medium. Cells were incubated with 10 μg/ml Dil-labeled AcLDL (Biomedical Technologies) for 4 h. For immunofluorescence microscopy, cells and sections were fixed with 4% PFAfor 10 min at room temperature (RT) or acetone for 5 min at -20^{o}C, permeabilized with 0.1% Triton

X-100 in PBS, and blocked with 3% goat serum. Subsequently, cells and frozen sections were incubated with primary and secondary antibodies for 1.5 h and 30 min, respectively. The nuclei were counterstained with Hoechest 33342 (5 μg/ml). Confocal image acquisition was performed using a Zeiss LSM 710 laser scanning microscope (Carl Zeiss). For whole mount staining, Matrigel plugs were processed as described previously (3). Briefly, whole plugs were fixed in methanol containing 25% DMSO for 24 h at 4^{o}C. After they were cut into 2 mm x 2 mm pieces, plugs were blocked in 3% goat serum for 3 h at 4^{o}C. The plugs were then incubated in primary and secondary antibodies for 20 h and 18 h at 4^{o}C, respectively. The images were acquired with a Zeiss Lightsheet Z.1 (Carl Zeiss) and analyzed using Imaris 3.1 software (Bitplane).

Hematoxylin/eosin staining. Frozen sections of the ischemic adductor muscles were fixed with 4% PFA for 10 min at RT and then stained with hematoxylin and eosin (both from Sakura Finetek) for 3 min each. After cleared with ethanol followed by Clear Plus (Falma), samples were observed using a light microscope (Nikon).

Capillary-like structure formation assay. 2 x 10^4 cells were seeded on 96-well flat-bottom plates coated with 30 μl Matrigel (BD Biosciences) and cultured in EGM-2 medium. Eighteen hours after incubation, capillary-like structures were observed under a BZ-8000 fluorescence microscope (Keyence). To investigate lumen formation, capillary-like structures were fixed with 4% PFA for 10 min at RT and subjected to immunofluorescence staining as described above.

Genomic DNA analysis. Genomic DNA of HFL-ECs cells was extracted using a

Wizard SV Genomic DNA Purification System (Promega) according to the manufacturer's instructiosn. DNA (15 ng) was subjected to PCR with Herculase II Fusion Enzyme (Agilent Technologies). PCR products were separated through 1.5% or 2% agarose gel, stained with ethidium bromide, and visualized using an UV transilluminator. Gene-specific primer pairs are listed in Table S2.

Western blot analysis. Cell lysates were prepared in 1% NP-40 lysis buffer containing protease inhibitor cocktail (Nacalai). Proteins were resolved on 12% SDS-PAGE gels and transferred to PVDF membranes (Millipore) by wet-transfer. Membranes were blocked for 1 h in PBS that contains 5% non-fat dry milk and 0.1% Tween 20 and then incubated with primary and secondary antibodies for 2 h and 1 h, respectively. Immunoreactivity was detected by chemiluminescence (Nacalai). The membranes were treated with stripping buffer (Nacalai) and then re-probed with anti-β-actin antibody. Protein expression levels were determined by measurement of band densities using ImageJ software.

Gene expression microarray analysis. Total RNA (300 ng per sample) was hybridized to a SurePrint G3 Human GE Microarray (8 x 60K) (Agilent Technologies) for 17 h and scanned using an Agilent Microarray Scanner. The relative hybridization intensities and background hybridization values were calculated using a Feature Extraction Software version 10.7.3.1 (Agilent Technologies). The raw signal intensities and flags for each probe were calculated from the hybridization intensities and spot information according to the procedures recommended by the manufacturer using the Flag criteria in GeneSpring12 (Agilent Technologies) in order to eliminate large negative or missing

values in the normalized data. The raw signal intensities of two samples were $\log_2$-transformed and normalized using the 50th percentile-shift and baseline transformed to the median of all samples. Differential expression was defined as a minimum 2-fold change and multiple testing-corrected $P < 0.01$ by one-way ANOVA. The resulting probes were used for hierarchical clustering using GeneSpring12. Microarray data analysis was supported by BIO MATRIX RESEARCH. Original data were uploaded to the Gene Expression Omnibus database (accession number GSE48980).

Flow cytometric analysis and cell sorting. Cells were detached using trypsin/EDTA (Nacalai) or Accutase (Sigma), resuspended in PBS-containing 2% FBS and 2 mM EDTA, and then stained with fluorochrome-leveled mAbs for 20 min on ice. Living cells were identified by 7-AAD exclusion and then analyzed for cell surface marker expression using a FACSCant II (BD). Fifteen and 25 days after lentivirus gene transduction, cells were labeled as described above and sorted using a FACSAria II (BD). Collected events were analyzed using FlowJo software (Tree Star).

***In vivo* Matrigel plug assay.** A total 1 x 10^6 cells were resuspended in 500 μl growth factor-reduced Matrigel (BD) supplemented with 300 ng/ml human bFGF and 10 U/ml heparin (Novo Nordisk Pharma) and injected subcutaneously into the abdominal flanks of 7 to 9-week-old male NOD.CB17-*Prkdc*^{scid}/J (NOD SCID) mice (Charles River) (3). Twenty-eight days later, the Matrigel plugs were removed using a wide excision in the abdominal wall, including the skin and all muscle layers, fixed with 4% PFA for 4 h at RT, and then incubated in 30% sucrose for another 18 h at RT. The Matrigel plugs were

embedded in 4% carboxymethylcellulose for storage at -80^{o}C until use. Frozen sections were sliced to a thickness of 20 μm using a cryostat (Carl Zeiss). For whole mount staining, Matrigel plugs were removed 42 days after the implantation and then processed as described above. The study protocol was approved by the Institutional Animal Care and Use Committee of Keio University (Protocol number 13016-(0)). Mice were randomly chosen for each experimental group, and no blinding was used.

Hind-limb ischemic mouse model. Nine- to 10-week-old male CAnN.Cg-*Foxn1*^{nu}/CrlCrlj (BALB/c-nu) mice (Charles River) were anesthetized using isoflurane (5% in 100% oxygen for induction, 1-2% in 100% oxygen for maintenance) supplied from animal anesthesia equipment (Model TK-5, Bio Machinery). Unilateral hind-limb ischemia was created by occluding the proximal portion of the femoral artery and the distal portion of the saphenous artery with an electric coagulator (Vetroson, V-10 Bi-polar, Electrosurgical Unit, Summit Hill Laboratories). Immediately after the surgery, mice were transplanted with 5 x 10^5 cells of HAFs (n = 10) or ETVECs (n = 6) at three equally spaced points on the adductor muscle of the ischemic thigh. Another group consisted of mice injected with 15 μl PBS (n = 10). Fourteen days after the transplantation, the blood flow in the lower limbs was determined as described below, and then mice were euthanized. Ischemic and non-ischemic adductor muscles of the mice were dissected and embedded in an OCT compound (Sakura Finetek). Frozen sections (7 μm thick) were subjected to immnunofluorescence staining. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health. In addition, the study protocol was approved by the Institutional Animal Care and Use Committee of Kurume University

School of Medicine. Sample size was chosen according to previous experience conducting the similar experiments (4). Mice were randomly chosen for each experimental group, and no blinding was used.

Laser Doppler imaging analysis. This was performed as described previously (5). Briefly, 14 days after transplantation, the blood flow in both limbs of the mice was determined using a laser Doppler blood flow imager MoorLDI™-Mark 2 (Laser Doppler Perfusion Imager System, MoorLDI^{TM}-Mark 2, Moor Instruments). Before scanning the blood flow, mice were placed on a heating pad at 37°C to minimize variations in temperature. The mean laser Doppler flux was analyzed on both limbs with software supplied by the manufacturer. To avoid the effects of light and temperature variables, hind-limb blood flow was expressed as the ratio of ischemic to non-ischemic hind-limbs.

Statistical analysis. Statistical analyses of all endpoints were performed using the two-sided Student's *t*-test or one-way ANOVA. The variance among the groups was estimated using F test or Bartlett test. All data are presented as mean ± SD. $P < 0.05$ was considered statistically significant.

1. De Haro L & Janknecht R (2002) Functional analysis of the transcription factor ER71 and its activation of the matrix metalloproteinase-1 promoter. *Nucleic acids research* 30(13):2972-2979.
2. Hollier, B.G. et al. (2012) FOXC2 expression links epithelial-mesenchymal transition and stem cell properties in breast cancer. *Cancer Res*. 73(6): 1981-1992.

3. Laib, A.M. et al. (2009) Spheroid-based human endothelial cell microvessel formation in vivo. *Nature protocols* 4(8): 1202-1215.

4. Sasaki K, *et al.* (2006) Ex vivo pretreatment of bone marrow mononuclear cells with endothelial NO synthase enhancer AVE9488 enhances their functional activity for cell therapy. *Proceedings of the National Academy of Sciences of the United States of America* 103(39):14537-14541.

5. Taniguchi, K. et al. (2009) Suppression of Sproutys has a therapeutic effect for a mouse model of ischemia by enhancing angiogenesis. *PloS one* 4(5): e5467.

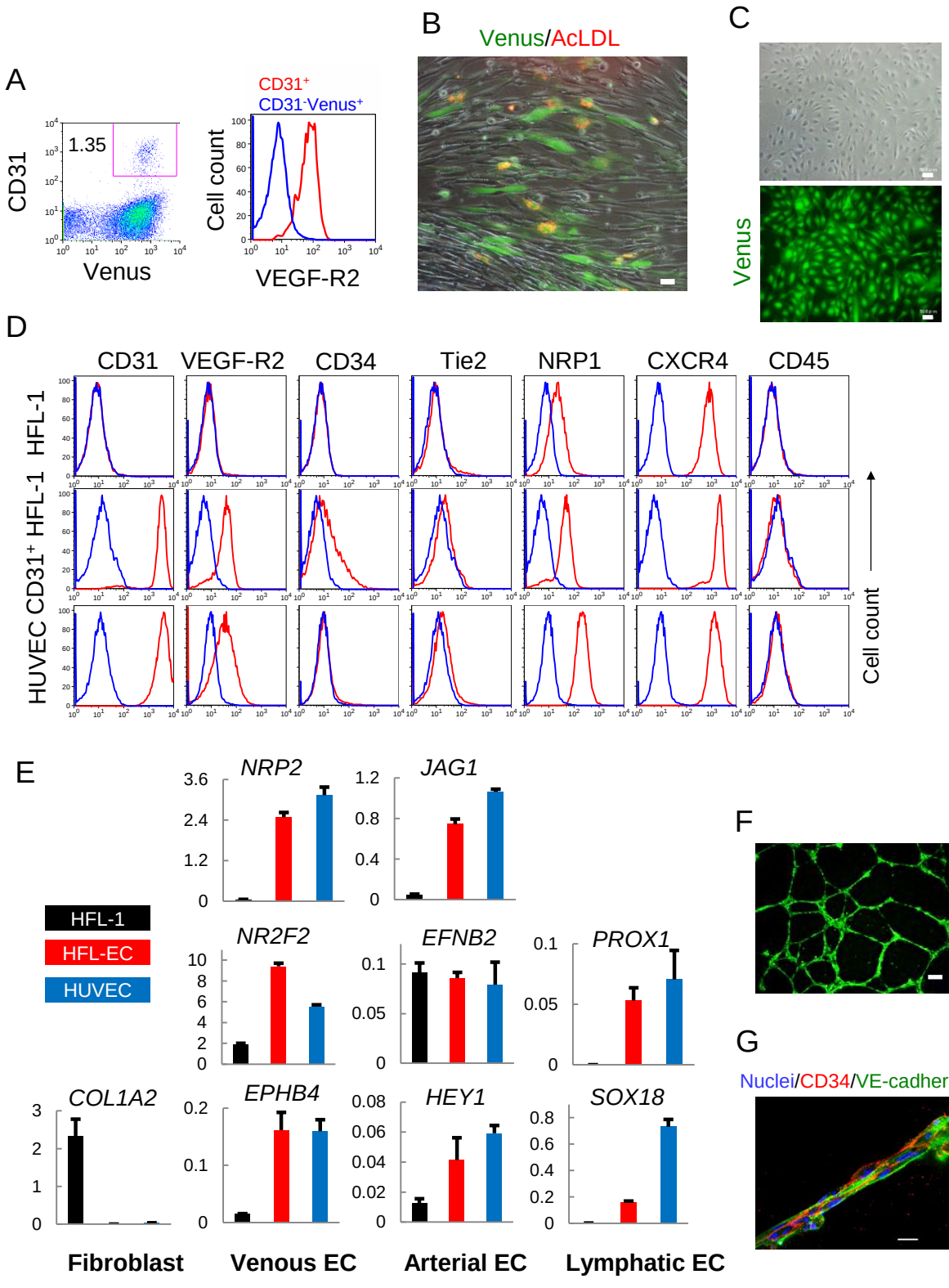
A
CD31
1.35
Venus
CD31+
CD31-Venus+
Cell count
VEGF-R2
B
Venus/AcLDL
C
Venus
D
CD31
VEGF-R2
CD34
Tie2
NRP1
CXCR4
CD45
HUVEC CD31+ HFL-1
HFL-1
Cell count
E
NRP2
JAG1
HFL-1
HFL-EC
HUVEC
NR2F2
EFNB2
PROX1
COL1A2
EPHB4
HEY1
SOX18
Fibroblast
Venous EC
Arterial EC
Lymphatic EC
F
G
Nuclei/CD34/VE-cadherin

Fig. S1. Screening for EC-inducing transcription factors. (*A*) $CD31^+VEGF\text{-}R2^+$ HFL-1 cells at 14 days after infection with a pool of 18 TF lentiviruses. The result is reported as a percentage of $CD31^+$ cells in the $Venus^+$ cells. (*B*) AcLDL-uptake of $Venus^+$ HFL-1 cells. Fourteen days after the infection with a pool of 18 TF lentiviruses, HFL-1 cells were treated with Dil-AcLDL for 4 h and then investigated using a fluorescence microscope. Scale bar, 50 μm. (*C*) Purified day 28 $CD31^+$ HFL-1 cells showed cobblestone-like morphology. The cells are all Venus-positive. (*D*) Original HFL-1 cells, sorted $CD31^+$ HFL-1 cells, and HUVECs were subjected to flow cytometric analysis. Red and blue lines indicate targets and isotype controls, respectively. (*E*) Quantitative RT-PCR was performed with HFL-ECs at 28 days after the 18 TF lentivirus infection. HFL-1 cells and HUVECs were used as negative and positive controls, respectively. Gene expression levels relative to *HPRT1* (mean ± SD; triplicate). (*F*) Capillary-like structure formation on Matrigel-coated plates. The structures are Venus-positive. Scale bar, 300 μm. (*G*) Lumen formation in the capillary-like structures on Matrigel-coated plates. Scale bar, 20 μm. Data are representative of four independent cell cultures.

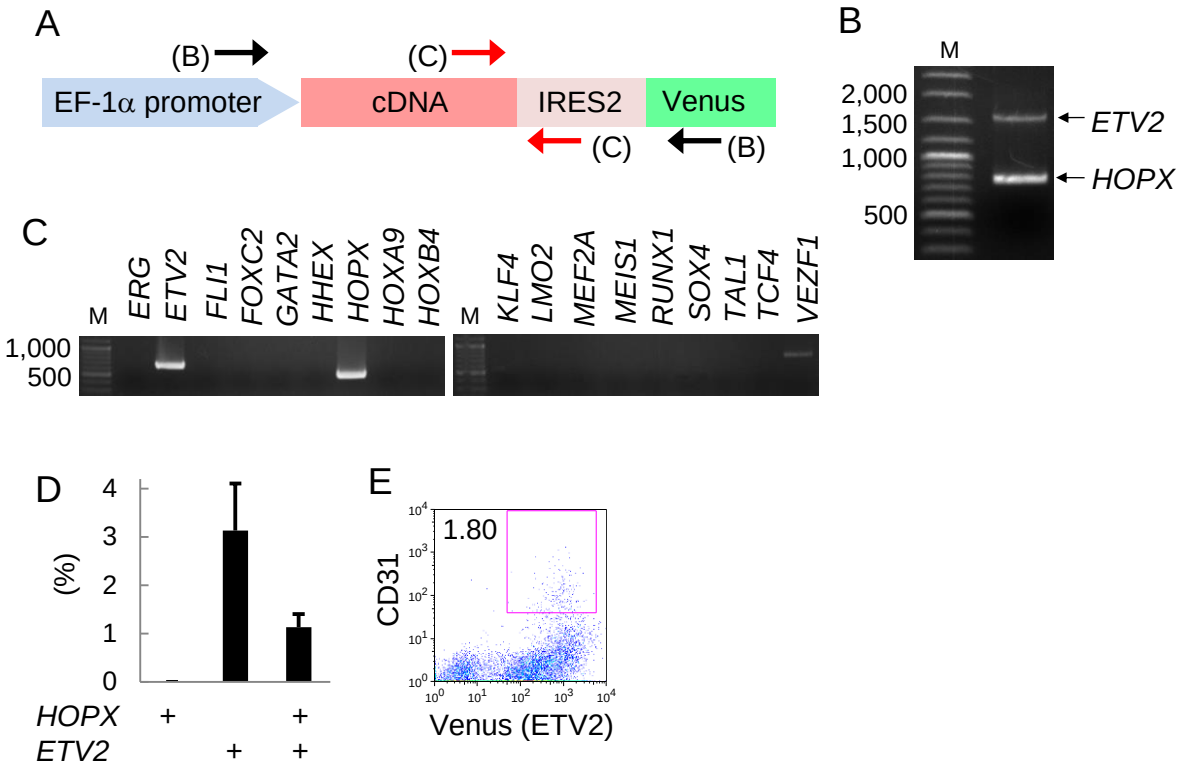

Fig. S2. *ETV2* is integrated into genomic DNA of HFL-ECs. (*A*) Scheme of part of the lentivirus vector and the positions that the two PCR primer pairs bind. (*B* and *C*) PCR analysis of genomic DNA from HFL-ECs. Each figure corresponds to the PCR primer pair depicted in Fig. S2A. M, size marker. (*D*) Percentages of $CD31^+$ cells in the $Venus^+$ HFL-1 cells at 14 days after the indicated factor transduction. Data are presented as mean ± SD; n = 4 cultures. (*E*) $CD31^+$ cells from NB1RGB cells at 14 days after *ETV2* transduction. The result is reported as a percentage of $CD31^+$ cells in the $Venus^+$ cells. The dot plot was represented by gates on $7\text{-}AAD^-$ $Venus^+$ cells. Data are representative of three experiments (*B* and *C*) and four independent cell cultures (*E*).

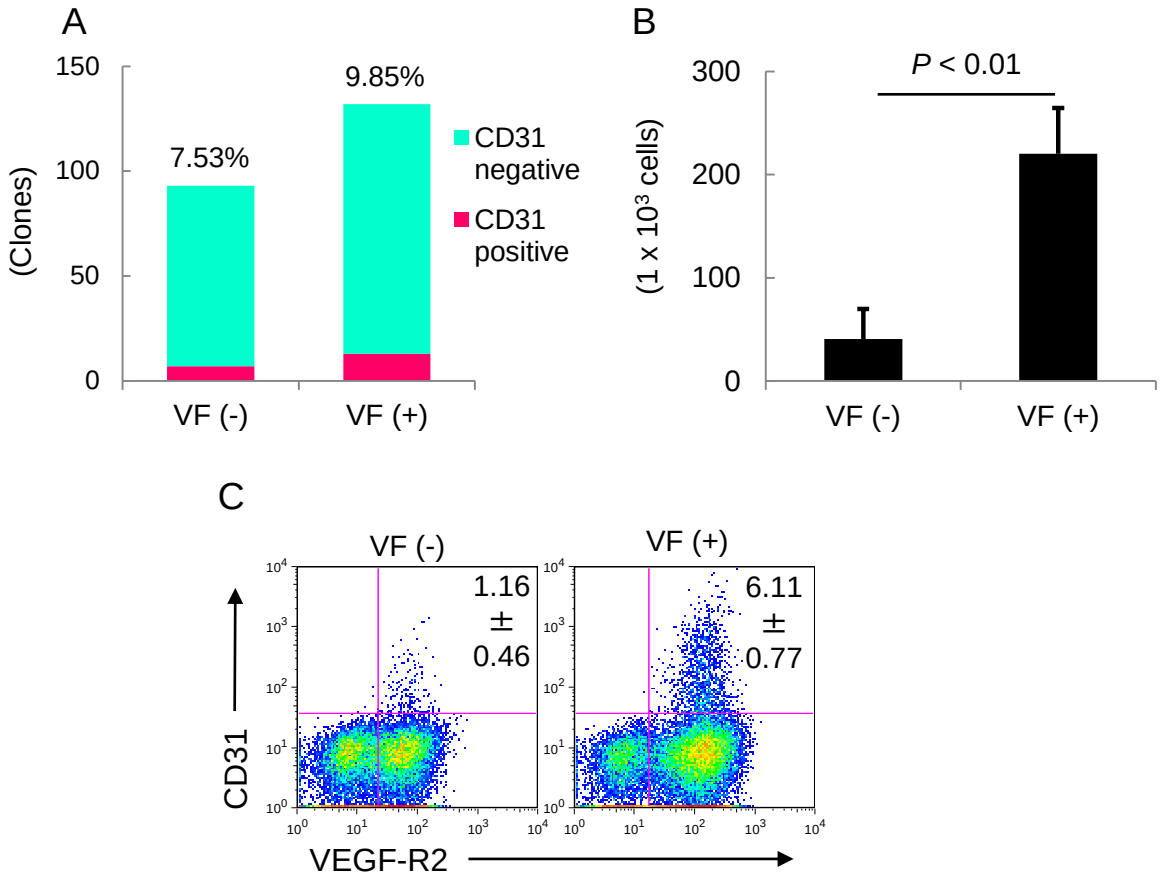

Fig. S3. VEGF and bFGF supplementation improves the ETVEC induction from *ETV2*-transduced HAFs. *ETV2*-transduced HAFs were cultured in ordinary EGM-2 medium (VF (-)) or that supplemented with VEGF and bFGF (both 10 ng/ml) (VF (+)) for 15 days. (*A*) Numbers of CD31-positive and -negative clones. Numbers above the bars indicate the percentage of CD31-positive clones in the total clones. (*B*) Absolute numbers of ETVECs. Two-sided Student's *t*-test. (*C*) Percentages of ETVECs. The dot plots were represented by gates on 7-AAD^{-}Venus^{+} cells. Data are representative of four independent cell cultures (mean ± SD; $n = 4$ cultures).

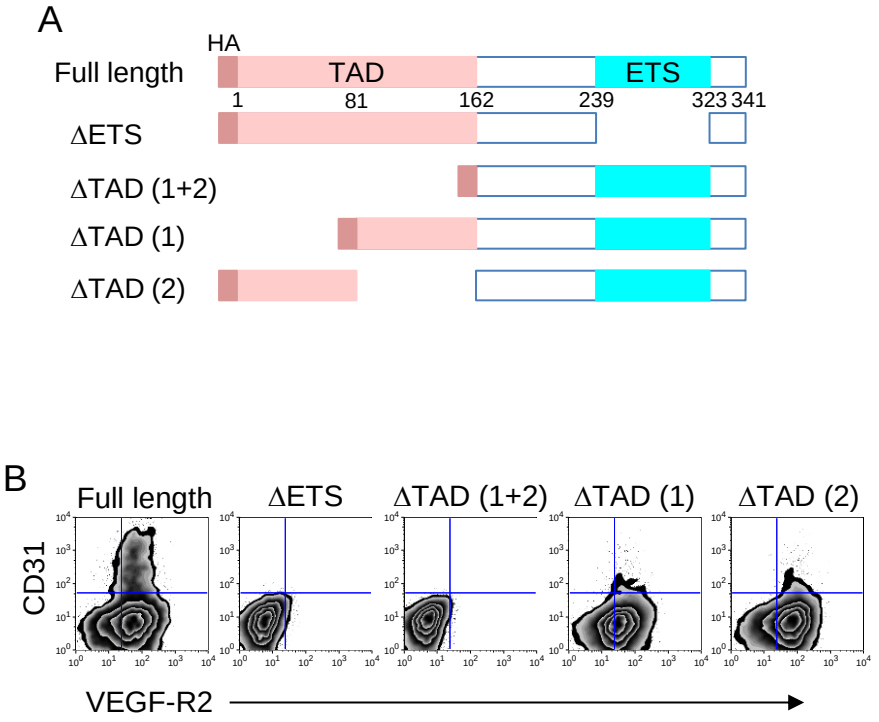

Fig. S4. Both the TAD and the ETS domain of ETV2 are essential for the EC induction. (*A*) Schematic representation of the ETV2 truncations. (*B*) HAFs at 15 days after the infection were subjected to flow cytometric analysis. The zebra plots are represented by gates on 7-AAD$^-$ Venus$^+$ cells. Data are representative of three independent cell cultures.

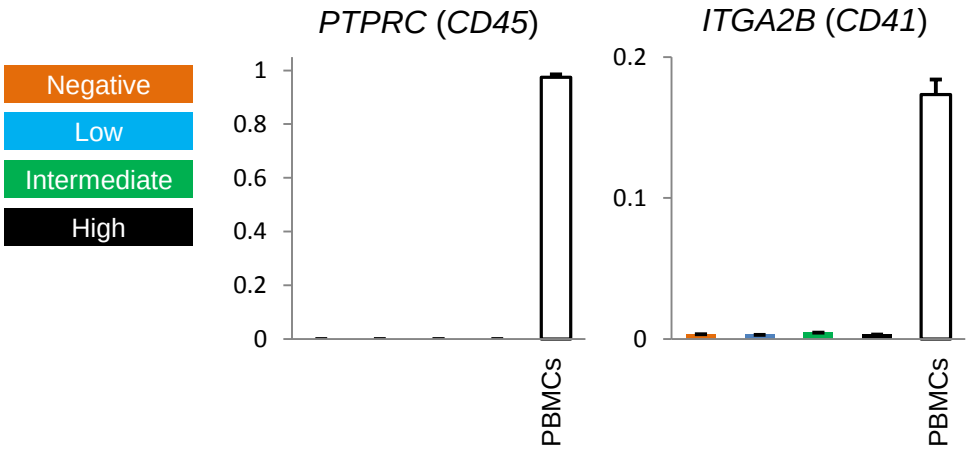

Fig. S5. *ETV2*-transduced HAFs did not express hematopoietic cell markers. HAFs at 14 days after *ETV2* infection were divided into the four populations, and then these populations were subjected to quantitative RT-PCR. PBMCs were used for a positive control. Gene expression levels relative to *HPRT1* (mean ± SD; triplicate).

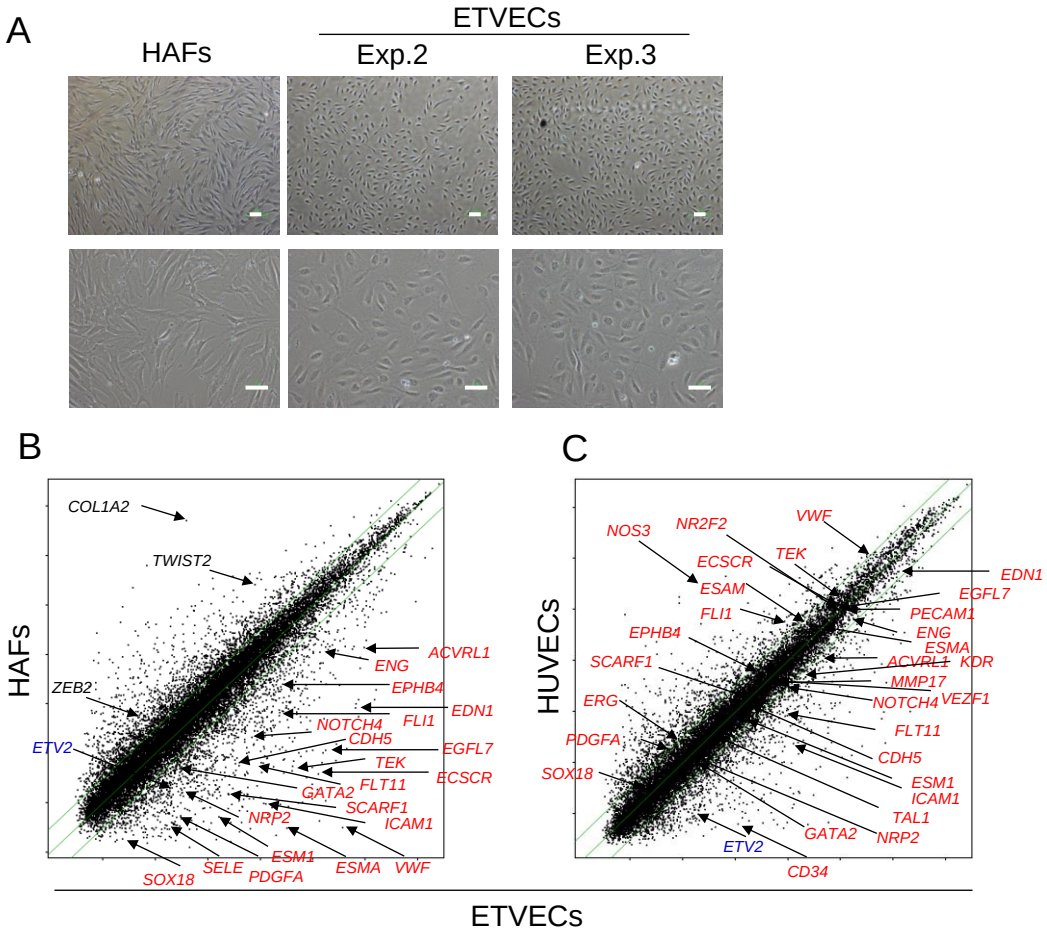

Fig. S6. *ETV2* induces expression of the EC-specific genes from HAFs. (*A*) HAFs and day 32 ETVECs were observed using a light microscope. ETVEC Exp.2 and Exp.3 were established from distinct skin donors. Top, low magnification images; bottom, high magnification images. Scale bar, 50 μm. (*B* and *C*) Scatter plots of the DNA microarray data. Red and black letters indicate endothelial- and fibroblast-specific genes, respectively. *ETV2* is indicated in blue.

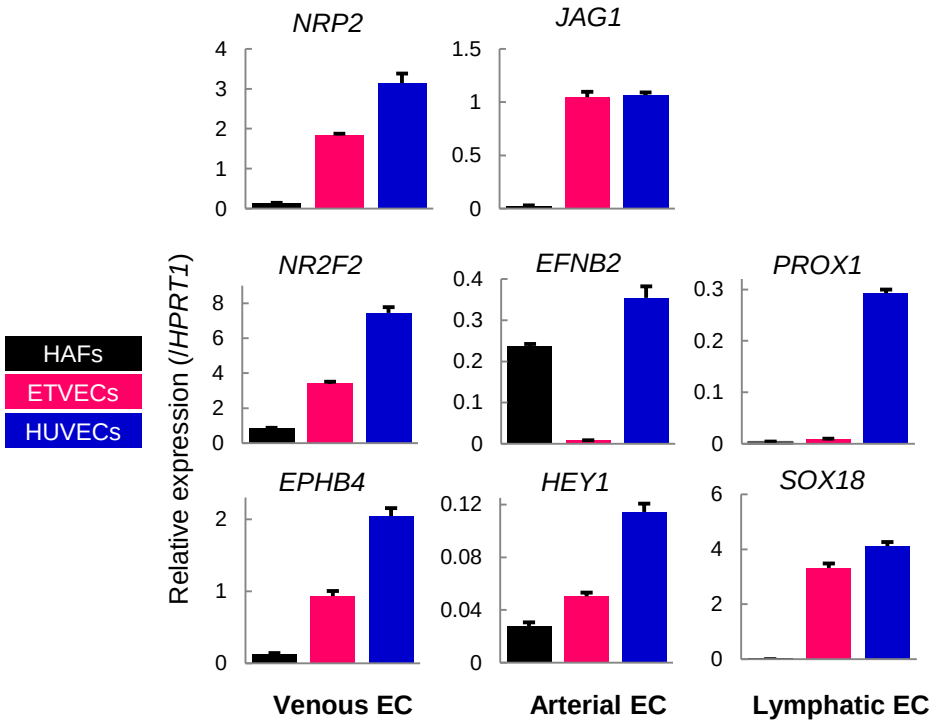

Fig. S7. ETVECs weakly but preferentially express the venous EC marker genes. Quantitative RT-PCR was performed with ETVECs. HAFs and HUVECs were used as negative and positive controls, respectively. Gene expression levels relative to *HPRT1*. Data are representative of three independent cell cultures (mean ± SD; triplicate).

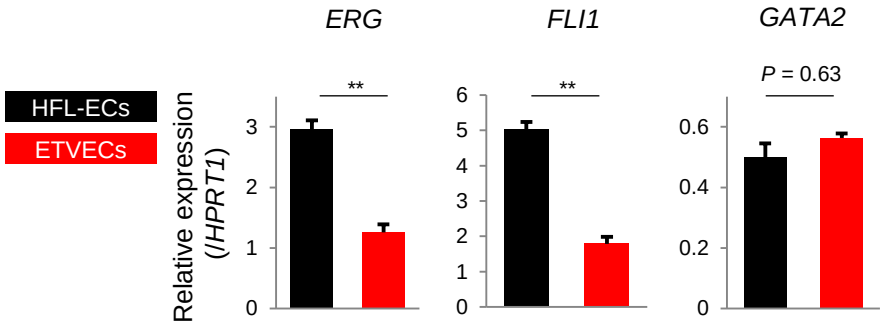

Fig. S8. HFL-ECs express higher levels of *ERG* and *FLI1* than ETVECs do. Quantitative RT-PCR was performed with HFL-ECs and ETVECs. Gene expression levels relative to *HPRT1*. Data are representative of three independent cell cultures (mean ± SD; triplicate). ** $P < 0.01$ (two-sided Student's *t*-test).

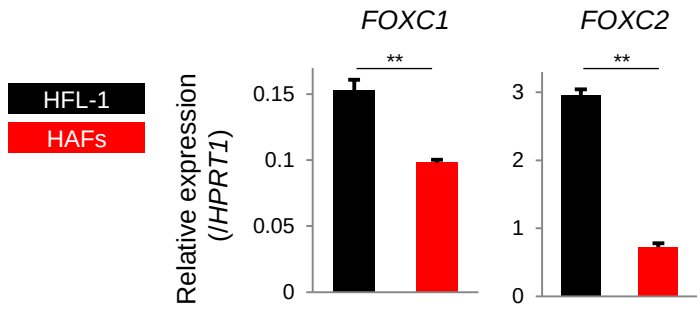

Fig. S9. HFL-1 cells express higher levels of *FOXC1* and *FOXC2* than HAFs do. Quantitative RT-PCR was performed with HFL-1 cells and HAFs. Gene expression levels relative to *HPRT1*. Data are representative of three independent cell cultures (mean ± SD; triplicate). ** $P < 0.01$ (two-sided Student's *t*-test).

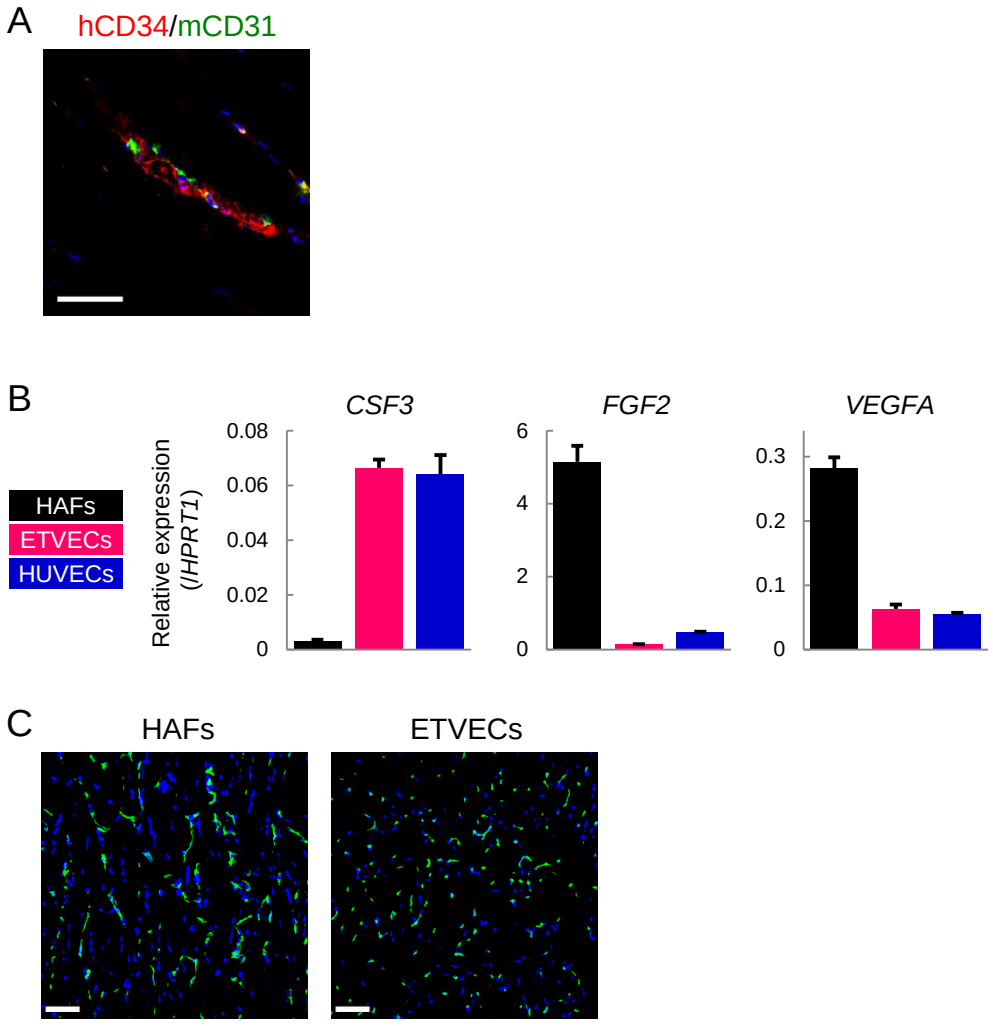

Fig. S10. ETVECs form functional vasculature in the ischemic muscles. (*A* and *C*) Adductor muscles of the ischemic hind-limbs at 14 days after the cell transplantation. ETVECs form vasculature in the ischemic muscles (*A*). Murine ECs incorporate into the ischemic muscles (*C*). Images indicate the same microscopic fields as those in Fig. 5*K*. Immunofluorescence images show mouse CD31 in green (Hoechest 33342 in blue). In all images, scale bars, 50 μm. Data are representative of five independent experiments. (*B*) Quantitative RT-PCR was performed with HAFs, ETVECs, and HUVECs. Gene expression levels relative to *HPRT1*. Data are representative of three independent cell cultures (mean ± SD; triplicate).

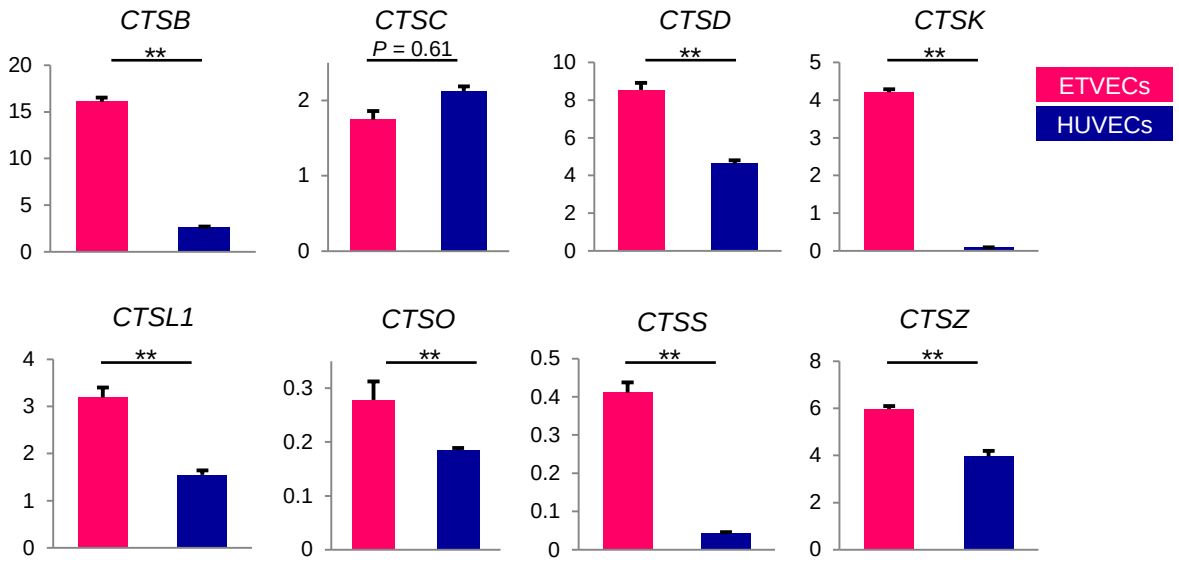

Fig. S11. ETVECs express a large series of cathepsin mRNA more than HUVECs. Quantitative RT-PCR was performed with ETVECs and HUVECs. Gene expression levels relative to *HPRT1*. Data are representative of three independent cell cultures (mean ± SD; triplicate). ** $P < 0.01$ (two-sided Student's *t*-test).

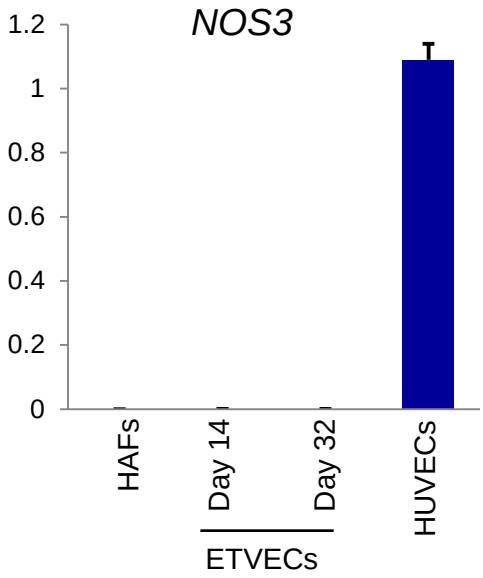

Fig. S12. ETVECs do not express *NOS3* mRNA. Quantitative RT-PCR was performed with HAFs, ETVECs, and HUVECs. Gene expression levels relative to *HPRT1*. Data are representative of three independent cell cultures (mean ± SD.; triplicate).

Gene	Gene Bank	PCR product (bp)	
		(*B*)	(*C*)
ERG	NM_001136155.1	1989	910
ETV2	NM_014209.2	1580	628
FLI1	NM_002017.4	1398	741
FOXC2	NM_005251.2	2070	515
GATA2	NM_001145661.1	1992	772
HHEX	NM_002729.4	1364	770
HOPX	NM_001145459.1	771	467
HOXA9	NM_152739.3	1378	661
HOXB4	NM_024015.4	1305	968
KLF4	NM_004235.4	1991	477
LMO2	NM_005574.3	1026	746
MEF2A	NM_005587.2	2043	814
MEIS1	NM_002398.2	1722	702
RUNX1	NM_001754.4	1994	783
SOX4	NM_003107.2	1981	508
TAL1	NM_003189.2	1556	784
TCF4	NM_001083962.1	2567	722
VEZF1	NM_007146.2	2115	850

Table S1. Transcription factors screened for direct conversion of human fibroblasts into ECs and their PCR product size. (*B*) and (*C*) are correspond to the primer pairs indicated in Fig. S2*A*.

(*B*)	Forward		GGCCAGCTTGGCACTTGATGTAATTCTCCTTG
	Reverse		CCACAACTATCCAACTCACAACGTGGCACTG
(*C*)	*ERG*	Forward	CAGTCGAAAGCTGCTCAACCATCTCCTTC
	ETV2		CAAAACTAACCACCGAGGTCCCATTCAGC
	FLI1		GTTCAAAATGACGGACCCCGATGAGGTG
	FOXC2		GTATCTCAACCACAGCGGGGACCTGA
	GATA2		CTGTGCAATGCCTGTGGCCTCTACC
	HHEX		GACGGTGAACGACTACACGCACGC
	HOPX		CACAGAGGACCAGGTGGAAATCCTGGAG
	HOXA9		CCACGCTTGACACTCACACTTTGTCCC
	HOXB4		CGAGGAATATTCACAGAGCGATTACCTACCCAG
	KLF4		ATCTCAAGGCACACCTGCGAACCCA
	LMO2		GTCCTCGGCCATCGAAAGGAAGAGC
	MEF2A		GTGTACTCAGCAATGCCGACTGCCTAC
	MEIS1		GTGATGGCTTGGACAACAGTGTAGCTTCC
	RUNX1		CAACCCTCTCTGCAGAACTTTCCAGTCGA
	SOX4		GACGACGAGTTCGAAGACGACCTGC
	TAL1		CCACCAACAATCGAGTGAAGAGGAGACCTTC
	TCF4		CCTGCAAGACACGAAATCTTCGGAGGAC
	VEZF1		GAAGAGACCAGTAACCAAAAGCAGCAGCAG
	Reverse		CAAAGGGTCGCTACAGACGTTGTTTGTCTTC

Table S2. PCR primers to detect factors integrated into HFL-EC genome. (*B*) and (*C*) are correspond to the primer pairs indicated in Fig. S2*A*.

Ag	Ab label	HAFs	ETVECs
Mouse IgG1	APC	8.06 ± 1.96	3.71 ± 0.25
VEGF-R2		8.96 ± 1.02	57.43 ± 15.92**
CD34		8.00 ± 1.99	701.00 ± 23.90 #**
Tie2		9.54 ± 1.59	44.50 ± 11.16**
NRP1		22.25 ± 7.69*	8.06 ± 2.13*
Mouse IgG1	PE/Cy7	7.21 ± 2.34	10.57 ± 1.17
CD31		7.59 ± 2.64	1,624.00 ± 303.45**
Endoglin		8.85 ± 2.67	274.00 ± 123.98*
CXCR4		8.44 ± 2.78	25.63 ± 2.35**
CD45		7.89 ± 1.89	9.12 ± 1.20

Table S3. Mean fluorescence intensity of surface molecule expression on HAFs and ETVECs. HAFs and day 32 ETVECs were subjected to flow cytometric analysis. #, All of the ETVECs showed a bimodal peak of CD34 expression. Data are presented as mean ± SD ($n = 4$ cultures). * $P < 0.05$, ** $P < 0.01$ (two-sided Student's t-test) compared to each labeled mouse IgG1.

Gene	Sequence	
CDH5	Forward	AGACCACGCCTCTGTCATGTACCAAATC
	Reverse	CACGATCTCATACCTGGCCTGCTTC
CFS3	Forward	AGCCAACTCCATAGCGGCCTTT
	Reverse	CCAGCTGCAGTGTGTCCAAGGT
COL1A2	Forward	TTGTTGCTGAGGGCAACAGCAGGTT
	Reverse	AAGGGCAGGCGTGATGGCTTATTTGT
CTSB	Forward	ATCCACACCAATGCGCACGTCAG
	Reverse	AGCTTCAGCAGGATAGCCACCATTACA
CTSC	Forward	TGATGACCTTGGCAATTCTGGCCATTTC
	Reverse	TGGTCACCTTGCTGCCCTCTTCTTTA
CTSD	Forward	CCATTCCCGAGGTGCTCAAGAACTACA
	Reverse	TTGGAGGAGCCCGTGTCGAAGA
CTSK	Forward	AACCCAACAGGCAAGGCAGCTAAA
	Reverse	GGCTTGCATCAATGGCCACAGAGA
CTSL1	Forward	TGCTGGTGGTTGGCTACGGATTT
	Reverse	GGTTTCTCCGGTCTTTGGCCATCTTTAC
CTSO	Forward	ACTCCACCGCCTTCTATGGAATAAATCAGT
	Reverse	TCTGCTGAGTATCTGGGAAACTTGGAAGG
CTSS	Forward	ATCGACTCAGACGCTTCCTATCCCTACA
	Reverse	GGGCCTTTATTGGCCACAGCTTCTT
CTSZ	Forward	TGGGAGGGAGAAGATGATGGCAGAAA
	Reverse	TGTGGTGTCCTGGTATTCGGCATAGA
EFNB2	Forward	TCCTCAACTGTGCCAAACCAGACCAA
	Reverse	AGGCCCTCCAAAGACCCATTTGATGT
EGFL7	Forward	AGTCGTTCGTGCAGCGTGTGT
	Reverse	CGGCGGTAGGCGGTCCTATAGAT
EPHB4	Forward	AAGAAAGTTTCGCAGCCGCTGGCTTT
	Reverse	TCATGTGCTGGACACTGGCCAAGATT
ERG	Forward	AACCATCTCCTTCCACAGTGCCCAAA
	Reverse	TTTGCAAGGCGGCTACTTGTTGGT
ETV2	Forward	AGGGAACAAGCTGGCAGGGCTTGAA
	Reverse	TCCAGCATGTCTCTGCTGTCGCTGT
FGF2	Forward	TGTGTGCTAACCGTTACCTGGCTATGA
	Reverse	GTGCCACATACCAACTGGTGTATTTCCTTG
FLI1	Forward	AGCGTTAGCAAATGCAGCAAGCTGGT
	Reverse	ATTGCCTCACATGCTCCTGTGTCCA
FOXC1	Forward	CACACCAGCGAACAGAATATCCCTCCAA
	Reverse	AGGCAAAGTGGAGGTGGCTCTGAATTA
FOXC2	Forward	ACAGCTACATCGCGCTCATCACCAT
	Reverse	ATGCTGTTCTGCCAGCCCTGCTTGTT
GATA2	Forward	CCACGACTACAGCAGCGGACTCTT
	Reverse	AGTTGACACACTCCCGGCCTTCT
HEY1	Forward	AAATGCTGCATACGGCAGGAGGGAAA
	Reverse	ATAACGCGCAACTTCTGCCAGGCAT
HPRT1	Forward	TGCTGAGGATTTGGAAAGGG
	Reverse	ACAGAGGGCTACAATGTGATG
ITGA2B (CD41)	Forward	GCAGAAGAAGGTGAGAGGGAGCAGAA
	Reverse	CATTCACAGTCCCAGGGCCATTGTT
JAG1	Forward	TTTGGAGCGACCTGTGTGGATGAGA
	Reverse	TGGTGATGCAAGGTCTCCCTGAAACT
KDR	Forward	AGCCATGTGGTCTCTCTGGTTGTGTATG
	Reverse	GTTTGAGTGGTGCCGTACTGGTAGGA
NOS3	Forward	TGACCCTCACCGCTACAACATCCT
	Reverse	CGTTGATTTCCACTGCTGCCTTGTCT
NR2F2	Forward	GGACCACATACGGATCTTCCAAGAGCAA
	Reverse	TTTCCTGCAAGCTTTCCACATGGGCT
NRP2	Forward	AGGAGCCCTGTGGTTGGATGTATGA
	Reverse	TGTCACTCTGCAGCCGCAAGAAAT

Gene	Sequence	
PECAM1	Forward	GGTCAGCAGCATCGTGGTCAACATAAC
	Reverse	TGGAGCAGGACAGGTTCAGTCTTTCA
PROX1	Forward	ACCCGTTATCCCAGCTCCAATATGCT
	Reverse	ATCGTTGATGGCTTGACGTGCGTA
PTPRC (CD45)	Forward	TAGGGACACGGCTGACTTCCAGATATGA
	Reverse	GTGTTGGGCTTTGCCCTGTCACAAATAC
RUNX1	Forward	AACAAGACCCTGCCCATCGCTTTC
	Reverse	GGTTCTTCATGGCTGCGGTAGCATTT
SOX18	Forward	TGAACGCCTTCATGGTGTGGGCAAA
	Reverse	CGCGTTCAGCTCCTTCCACGCTTT
T (brachyury)	Forward	GCGCTTCAAGGAGCTCACCAATGA
	Reverse	CGTTCACCTTCAGCACCGGAAACA
TAL1	Forward	ACCACCAACAATCGAGTGAAGAGGAGAC
	Reverse	CTGTTGGTGAAGATACGCCGCACAA
VEGFA	Forward	ACTTTCTGCTGTCTTGGGTGCATTGG
	Reverse	TTCGTGATGATTCTGCCCTCCTCCTT
VEZF1	Forward	GCAGCAGCAGCAACAACAACAACA
	Reverse	AGGTTTGGCACAGGTTAGCAGCTT
VWF	Forward	TCTCCGTGGTCCTGAAGCAGACATA
	Reverse	AGGTTGCTGCTGGTGAGGTCATT

Table S4. Gene-specific primers used for quantitative RT-PCR.